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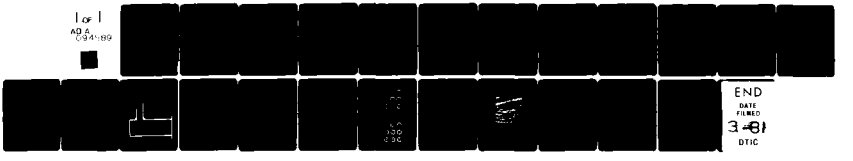

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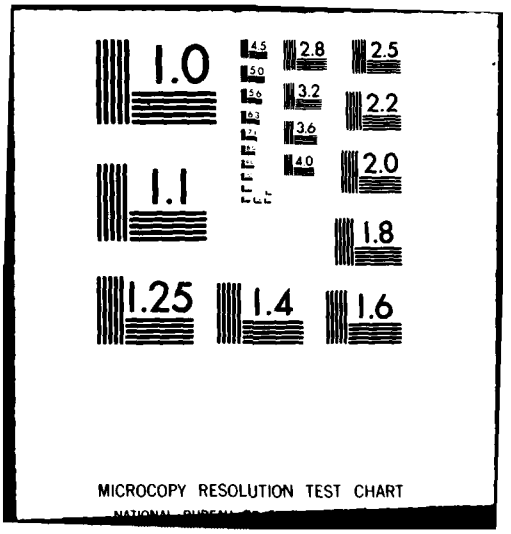
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MICROWAVE TEMPERATURE EFFECTS ON THE MURINE OCULAR LENS IN VITRO

Annual Summary Report

DR. JOHN R. TREVITHICK

June, 1980

September, 1979 - May, 1980

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Fort Detrick, Frederick, Maryland 21701

Grant No. DAMD-17-78-G-9449

University of Western Ontario
London, Ontario, Canada N6A 5C1

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER

2. GOVT ACCESSION NO. AD-A094889

3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle) (6)
Microwave and Temperature Effects on the Murine Ocular Lens In Vitro

5. TYPE OF REPORT & PERIOD COVERED (9)
Annual Summary Report.
~~Sept. 1, 1979-May 30, 1980~~
1 Sep 79-30 May 80.

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s) (10)
John R. Trevithick

8. CONTRACT OR GRANT NUMBER(s) (15)
DAMD17-78-G-9449, PHS-EY 927

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Department of Biochemistry
University of Western Ontario
London, Ontario, Canada N6A 5C1

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS (16) (17)
62771A 3E162771A805 00 005

11. CONTROLLING OFFICE NAME AND ADDRESS (11)
US Army Medical Research and Development Command, Fort Detrick, Frederick, MD 21701

12. REPORT DATE
June 1980

13. NUMBER OF PAGES

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) (12) (23)

15. SECURITY CLASS. (of this report)
Unclassified

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

approved for public release; Distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

Approved for public release; Distribution Unlimited

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Rat eye lens, microwave irradiation, pulsed and continuous waves, tissue culture, medium 199, elevated temperature, globular degeneration, cataract vitamin E prevention.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Rat ocular lenses were studied after fixation and critical point drying of the tissue by scanning electron microscopy (SEM) following exposures to elevated temperatures and/or microwave irradiation in a thermostatically controlled chamber. In this way, the temperature of the lens bathing medium was set independently of the temperature increase normally associated with application of microwave power. Irradiations were done at three final temperatures and three specific absorption rates (SAR) → cont.

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for two durations. These were accomplished at 915 MHz in WR975 waveguide with either pulsed (Pu) or continuous wave (CW) radiation of equal average power. The parameters of the (Pu) radiation were selected to maximize the production of thermoacoustic expansion. The results are summarized as follows:

1. Exposure of lenses in vitro to elevated temperatures for 1 hour followed by a 2-day incubation in tissue culture medium 199 (M199) produced predominantly subcapsular and epithelial defects at 39° C, while at 41° C, extensive globular degeneration was added. At 43° C and 45° C, the depth of globular degeneration increased proportionately. At 47° C and 50° C, large globules were produced.

2. Microwave pulsed irradiation in vitro at 37° C followed by a 2-day incubation in M199 produced globular bodies and subcapsular foam at the lowest SAR (120 mW/g) and shortest duration (5 min). At the intermediate SAR (400 mW/g) and 5 minute duration, holes in the lens fibers were added. At the highest SAR (1.0-1.2 W/g) and 5 minute duration, large globules and more holes were produced. In all cases, Pu produced more extensive damage than CW radiation.

3. Microwave irradiation at 39° C and 41° C followed by a 2-day incubation in M199 caused more damage at all SARs and durations than at 37° C. In general, increased temperature appeared to result in a similar effect to raising theSAR between 37C and 39° C. At 41° C deeper penetration of the globular degeneration towards the nuclear region was added. In all cases, Pu caused more damage than CW radiation for a given SAR and temperature.

4. Fixation of the lenses without incubation did not reveal any defect in the sham irradiated controls at 39° C. However, the same fixation following irradiation at 39° C with the highest SAR and either the 5 or 20 minute duration revealed subcapsular foam, curled epithelium, and holes in the fiber cells by SEM examination. Pu radiation appeared to be more effective than CW radiation of the same average power.

5. At a given temperature, increased duration appeared to substitute for lower SAR. For example, defects produced at 37° C with 400 mW/g for 20 minutes are comparable to those produced by 1.2 W/g and 5 minutes. A similar situation existed at 39° C. The total absorbed energy (joules) for these examples were comparable within 1 dB.

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Summary

The eventual aims of these experiments are to use intact rat lenses incubated *in vivo* in tissue culture medium to study the development of cataracts when the lenses are exposed to elevated temperatures and/or microwave irradiation by high-energy-pulsed microwaves, to establish (a) cataractogenic conditions for irradiation and (b) the mechanism of cataractogenesis in such lenses.

Intact rat lenses incubated in Medium 199 at 35.5°C can maintain their transparency for at least 9 days.

Rat ocular lenses were studied after fixation and critical point drying of the tissue by scanning electron microscopy (SEM) following exposures to elevated temperatures and/or microwave irradiation in a thermostatically controlled chamber. In this way, the temperature of the lens bathing medium was set independently of the temperature increase normally associated with application of microwave power. Irradiations were done at three final temperatures and three specific absorption rates (SAR) for two durations. These were accomplished at 915 MHz in WR975 waveguide with either pulsed (Pu) or continuous wave (CW) radiation of equal average power. The parametersof the (Pu) radiation were selected to maximize the production of thermoacoustic expansion. The results are summarized as follows:

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Foreword

A. List of Professional Personnel Employed on This Project

Principal Investigator	-	Dr. John R. Trevithick, Ph.D.
Research Associate	-	Dr. P. Jill Stewart-DeHaan, Ph.D.
Research Associate	-	Dr. William M. Ross, Ph.D.

B. Animal Care

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Science - National Research Council, U.S.A.

Introduction

Although a number of studies of microwave effects on the ocular lens *in vivo* have been performed[1,2] they have generally suffered from two disadvantages: 1) it has been difficult to separate the effects of heating from the effects due to the electromagnetic field; 2) it has generally been difficult to characterize any definitive precataractous changes in the lens.

In the experiments reported here, a system was devised for microwave irradiation of intact lenses *in vitro*[3] which permits the temperature of the lens bathing medium to be varied independently of the irradiating field, and by post-irradiation incubation in M199, which permits precataractous and cataractous changes to be detected by SEM. These changes may be compared to known changes involved in the course of cataractogenesis induced by high glucose levels in M199[4], or by temperature elevation alone.

Materials and Methods

Lenses with intact capsules were placed in M199 in culture tubes or in an apparatus in which they could be bathed in circulating bicarbonate or phosphate buffered saline (PBS) at controlled temperatures while simultaneously being subjected to microwave radiation. Intact lenses were exposed to elevated temperatures for periods of up to one hour, followed by incubation in M199 at 35.5° C for two days. Some lenses were pre- and post-exposure incubated in M199 with 1 μg/ml of α-tocopherol added in order to explore possible prophylactic effects for exposure to elevated temperatures.

Microwave radiation was delivered to the lens holder in two modes, Pu and CW. The lens holder is shown in the waveguide structure in Fig. 1 and the exposure system block diagram in Fig. 2. In the CW case, the signal was generated by a Hewlett Packard (HP) 8690B Sweep Oscillator with an HP 8699B plug-in, filtered by an HP 360B low pass filter to remove harmonics, amplified by an Amplifier Research 4W1000 amplifier and a Varian VZL-6943G1 TWT amplifier and then filtered again to remove harmonics and noise generated by the TWT. The power was adjusted to the desired level by setting the output level from the sweep oscillator. In the Pu case, the signal was generated by an Epsco PH40K and passed through a PAMTECK UTA1017 isolator to protect the pulsed source from excessive reflected power. In either case, the signal was passed through a coax-to-waveguide adaptor (Dielectric Communications C-42061-501). A reflectometer (Dielectric Communications D-40588-502) was used in conjunction with HP435A power meters to monitor forward and reflected powers. The impedance match to the lens holder was done using a triple stub tuner designed and built at Walter Reed. Typically, it was possible to adjust the tuner so that the reflected power was 15-18 dB below the forward power with the lens holder in place. The lens holder was located 1/4 guide wavelength from the waveguide shorting plate placing the lens holder in the maximum of the electric field. The vertical position of the lens holder was adjusted so that the lens was approximately at the center of the waveguide.

Average transmitted powers were 60 W, 20 W, 6 W, and zero W for the shams. The corresponding SARs (as determined by measuring temperature elevation at the lens site with the coolant flow off) were 1.0-1.2 W/g, 300-400 mW/g, 100-120 mW/g and zero respectively. The bath offset temperature to meet the final temperatures was 1.4--1.6°C at 60 W of average power (with coolant flow), 0.3-0.4° C at 20W and very close to zero at 6 W. The bathing medium was PBS. Flow rate was 600 ml/minute.

In the pulsed case, average power was set by variation of the pulse repetition rate. The peak power and pulse rise time were constant at about 20 KW and 0.1 to 0.5 μsec, respectively.

Lenses were fixed either immediately after irradiation or after the 2-day incubation in M199. After treatment with Karnovsky's fixative[5] for 48 hours at 4° C, the lenses were transferred to 0.1 M Na-cacodylate buffer, dehydrated in an alcohol series to acetone and critical point dried from CO_2. Each lens was quartered and attached to an SEM stub with silver daube paint, spatter-coated with gold-palladium and examined in a Hitachi HHS-2R SEM at an accelerating voltage of 20 KV.

RESULTS: Thermal Controls/Elevated Temperature

When unirradiated lenses were exposed to various temperatures for 1 hour, and then incubated at 35.5° C for 2 days in M199, then fixed, the transparency of the lens decreased progressively as the temperature of incubation increased, in the range of 37-50° C. However, after incubation at 60° C, no loss of transparency could be seen. Examination of the lenses by SEM revealed, at 37° C, no changes in the normal morphological features of the lens epithelial or fiber cells. At 39° C, the equatorial epithelial cells were not tightly joined to each other at the cell borders, which appeared swollen, were curled up from the underlying fiber cells, and had pitted surfaces; also at 39° C, equatorial fiber cells showed decreased interdigitation; in one area they were twisted and distorted and, at the equatorial region itself, showed incipient globular degeneration in a wedge-shaped ring (with the wedge apex towards the lens nucleus). At 41° C the lens epithelial cell surfaces were fibrous and necrotic, with many deep pits, and in many areas the underlying fiber cells were denuded of their epithelial coating; the fiber cells were extensively involved in globular degeneration which consisted of a much larger equatorial, wedge-shaped area (at which the maximum depth of globular degeneration was measured), and which now extended anteriorly and posteriorly to the poles of the lens (where a minimum depth of degeneration was measured). Pre- and post-incubation in vitamin E enriched medium prevented most of the globular degeneration associated with exposure to 41° C. At 43° and 45° C, no epithelial cells per se could be identified, and the depth of degeneration increased progressively.

An unusual feature seen first at 47° C and 50° C was the appearance of very large globules (up to 200 μ in diameter, 10 times the usual size). At 60° C there were no apparent differences in morphology from normal lenses, either in epithelial or fiber cells, presumably because the cells had been "fixed" by the extreme temperature. Only slight changes in morphology of nuclear lens fiber cells were detectable in lenses incubated at 45° C, as compared to those at 37° C. These data are consistent with our previous suggestion[3] that globular degeneration and opacity appear to be associated in a causal sense. The absence of globular degeneration in lenses exposed to 60° C, which results in heat fixation, appears to account for the lack of lenticular opacity.

RESULTS: Microwave Exposure

Exposure to Pu irradiation was performed under conditions optimized for thermoacoustic expansion (within the capabilities of the EPSCO PH40K pulsed source). Thermoacoustic transduction was verified by a third octave analysis of the radiated sound field. Such Pu radiation (Fig. 3a,b) resulted in more extensive damage to lenses than CW radiation at the same average power or equivalent temperature exposure at all temperatures tested. Even at 37° C where no change in morphology was observed in unirradiated samples, damage could be detected at SARs delivered to the lens of as little as 120 mW/g. The amount of damage appeared to increase roughly in proportion to the SAR and/or time of irradiation, a relationship which suggests reciprocity but which requires further testing. Features of the damage observed following Pu irradiation were: (1) the appearance of holes in the fixed, critical-point-dried lens fiber cells irradiated at temperatures as low as 37° C (Fig. 4a, b); (2) more extensive globular degeneration than observed in thermal controls at 39° C and 41° C for the same duration at identical temperatures; and (3) the appearance of large globules in the microwave irradiated lenses at 37° C, similar to those observed only at temperatures of 47° C and 50° C in unirradiated samples.

A comparison of lenticular damage caused by CW and Pu radiation at the same average power and final temperature revealed: (1) CW irradiation did produce damage at 37° C that was qualitatively similar to that produced by Pu, but, (2) less extensive damage was observed for CW than for Pu at all temperatures and all SARs; in particular, preliminary observations suggest that the threshold for holes and globular degeneration appeared to be lower for pulsed radiation; (3) both CW and Pu irradiation did produce holes in the lens fiber cells at 37° C for the higher SARs, but the holes were more numerous, larger in diameter, and at a greater depth relative to the capsule for Pu; and (4) a rough proportionality based on the extent of the damage was observed as power flux density was substituted for duration in both CW and Pu irradiation, a finding which will require further testing for confirmation.

In order to ascertain if the damage to the lens required the 2-day incubation for detection by SEM, several lenses were fixed immediately after Pu and CW irradiation. Such lenses showed rather large numbers of holes, which was greater for Pu than CW, similar in number to the holes found in the lenses incubated for 2 days following the irradiation. A similar analysis of the temperature controls where fixation took place immediately after exposure to elevated temperature, did not reveal the presence of holes in the lens fibers.

Discussion

The in vitro system developed for exposing lenses in medium to the stress of chemical compounds, temperature, and irradiation permits the precise manipulation of these factors, whereas, in vivo, only an approximation is possible. In addition, the various types of damage and their degree or extent are more readily and rapidly detectable and assessable; for example, incubation with 55.6 mM glucose in M199 results, within 2 days, in development of both globular degeneration and opacity, while the same process in the intact diabetic animal would require as much as 6-8 weeks to reach a similar degree of degeneration (unpublished observations).

Several observations in lenses exposed to elevated temperature alone are pertinent to and useful in the interpretation of the effects of microwaves on lenses incubated in a circulating medium of constant temperature. (1) The linear relationship between maximum depth of globular degeneration and temperature up to 50° C (Fig. 5) will be useful in interpolating additional effects of microwave irradiation on the isolated lens in terms of temperature equivalence. (2) The large globules found in microwave irradiated lenses at 37° C and in unirradiated lenses at 47° and 50° C are another morphological marker found in our studies of lenses incubated in vitro (only in lenses exposed to elevated temperature, but never in lenses exposed to other cataractogenic conditions, as diverse as elevated levels of sugars, steroids, hygromycin B or drugs such as cytochalasin D, colcemid, or vinblastin sulfate). This observation should also be useful in estimating the "temperature elevation equivalent effect" of additional microwave irradiation. (3) The observation that incubation with vitamin E enriched M199 prevented most of the cataractogenic globular degeneration in lenses heated to 41° C, indicates a possibility for both prevention of potential heat-induced cataracts and a rationale for treatment of individuals whose eyes are inadvertently exposed to large amounts of heat and/or infrared radiation. It is presently unclear whether the vitamin E acts as an antioxidant or scavenger of free radicals or on some other membrane activity such as fluidity. Preliminary observations on lenses exposed to either Pu or CW microwave radiation indicate that both types can apparently cause an additional effect - holes in the cell membranes observed after fixation and critical point drying - when compared to unirradiated controls at the same temperature. This effect appears to occur either concurrently with the irradiation or immediately afterwards, since it is found both in lenses fixed immediately after Pu irradiation as well as after the further 47 hour incubation. No SEM detectable defect was observed with temperature elevation alone without the 2-day incubation in M199.

Thermoacoustic expansion[6] at 37° C apparently results in large globules, an effect equivalent to a 10° C temperature elevation.

Comparison of the results of CW and Pu irradiation indicates that CW was not as effective as Pu in causing damage to the lens, possibly because of the additional mechanical stress resulting from thermoacoustic expansion caused by Pu microwaves. In that context, it is useful to recall the fact that adjacent fibers are mechanically connected by numerous regions of interdigitation along their lengths. Further, the thermal conductivity and specific heat of the lens fibers is anisotropic due to its large ratio of length to width and the different constitution of its materials in length (cytoplasm, cytoskeleton) and width (membrane). Thus, mechanical amplification can take place as would be the case with a bimetallic strip. CW irradiation did, however, also result in extensive globular degeneration after as little as 5 minutes at maximum SAR at 37° C, a temperature at which no degeneration would have been expected in the absence of microwaves. Microwave exposures at elevated temperatures for both CW and Pu radiation resulted in more extensive lenticular damage than irradiations at 37° C.

Acknowledgements

We thank Dr. Piro Kramar for helpful discussions. We also wish to acknowledge the excellent technical assistance of Mr. Leon S. Butler for performing the surgical procedures to remove the lenses. We thank Mr. Warren Foster for his contributions in designing and building the glass lens holders. Grants from the U.S. Army Medical Research and Development Command (DAMD-78-G-9449), the MRC of Canada, National Eye Institute (USA) (EY1927) and the E.A. Baker Foundation of the CNIB are acknowledged with thanks.

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Fig. 1. Diagram of exposure apparatus showing the waveguide structure and glass lens holder.

Lens Holder in Waveguide

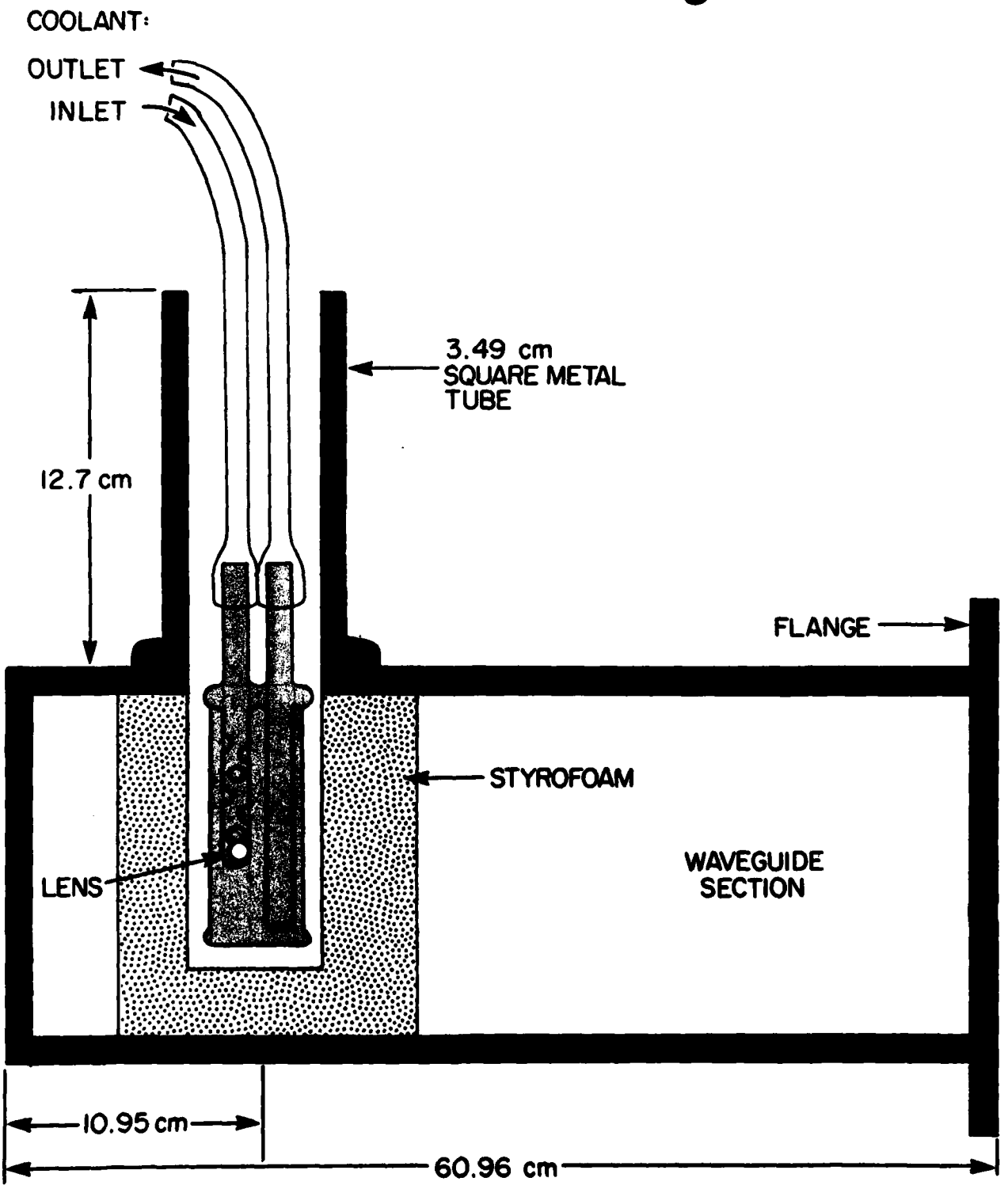
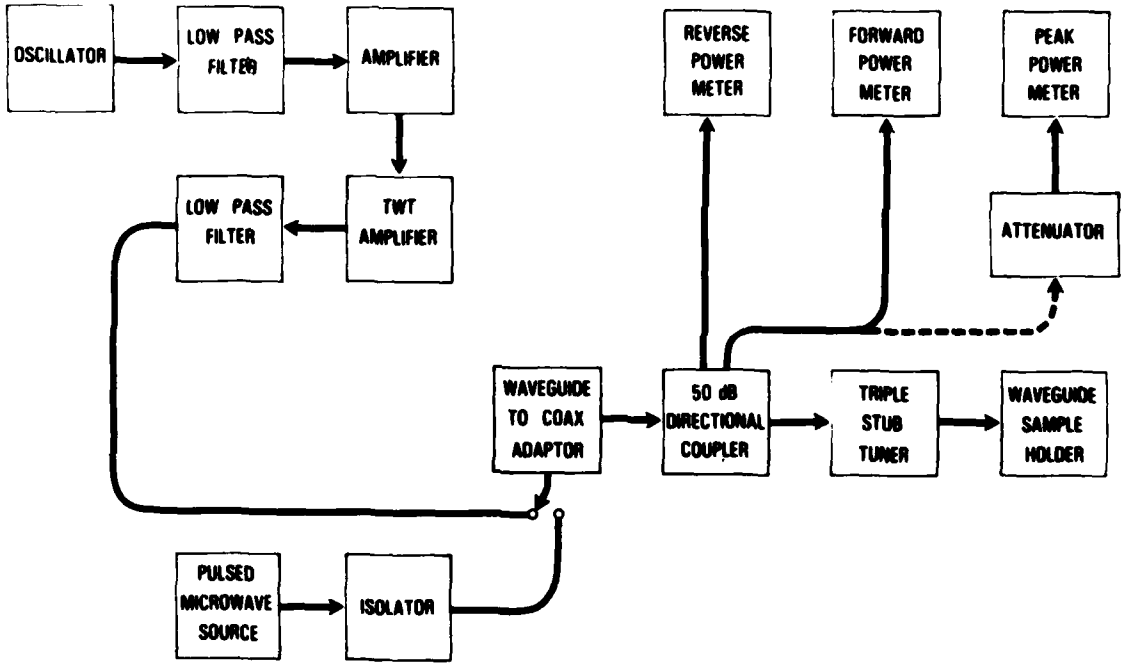


Fig. 2. Block diagram of exposure system.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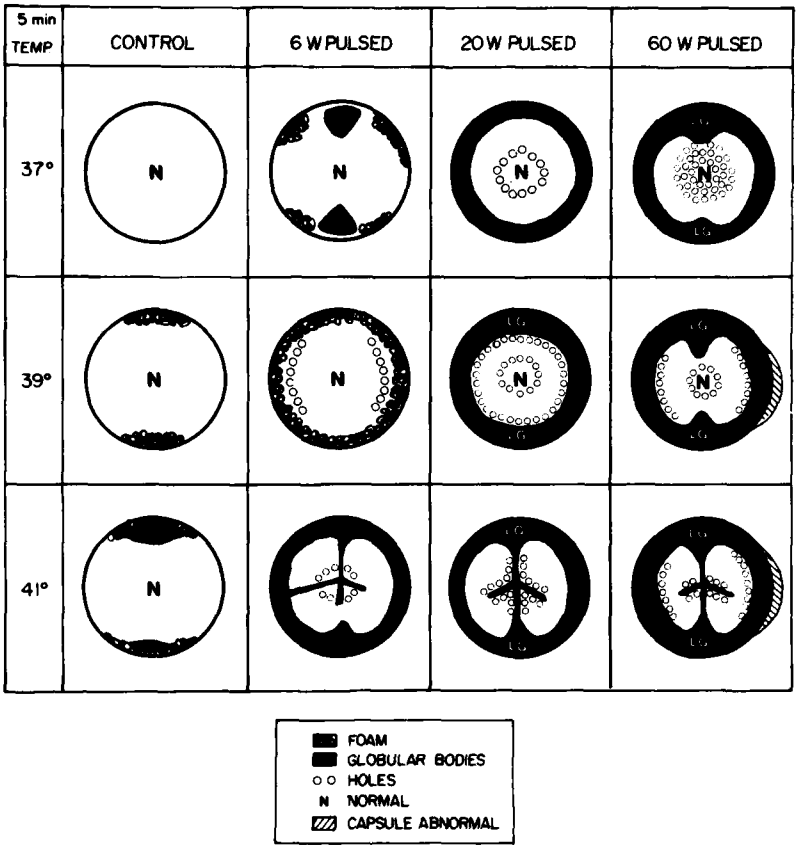
WAVEGUIDE TO COAX ADAPTOR
50 dB DIRECTIONAL COUPLER
TRIPLE STUB TUNER
WAVEGUIDE SAMPLE HOLDER
PULSED MICROWAVE SOURCE
ISOLATOR

Fig. 3. Microwave irradiation (915 MHz, pulsed) of rat lenses in vitro, showing extent of damage represented diagrammatically: foam, globular degeneration, etc. (a) exposure for 5 min. (b) exposure for 20 min. The damage was greater with irradiation then in unirradiated controls, and with greater duration of exposure for the same dose rate.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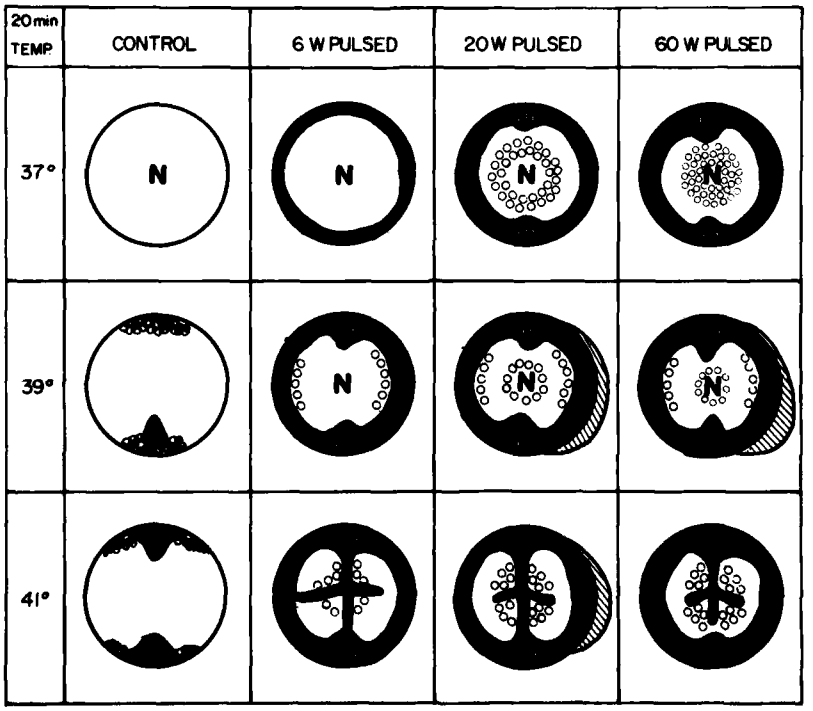
20 min TEMP
CONTROL
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Fig. 4. Lenticular fiber cell damage by microwave irradiation detected by SEM after fixation and critical point drying: (a) normal lens fiber cell (5000 x); (b) irradiated fiber cells. Note presence of holes in surface of fiber cells (5000 x).

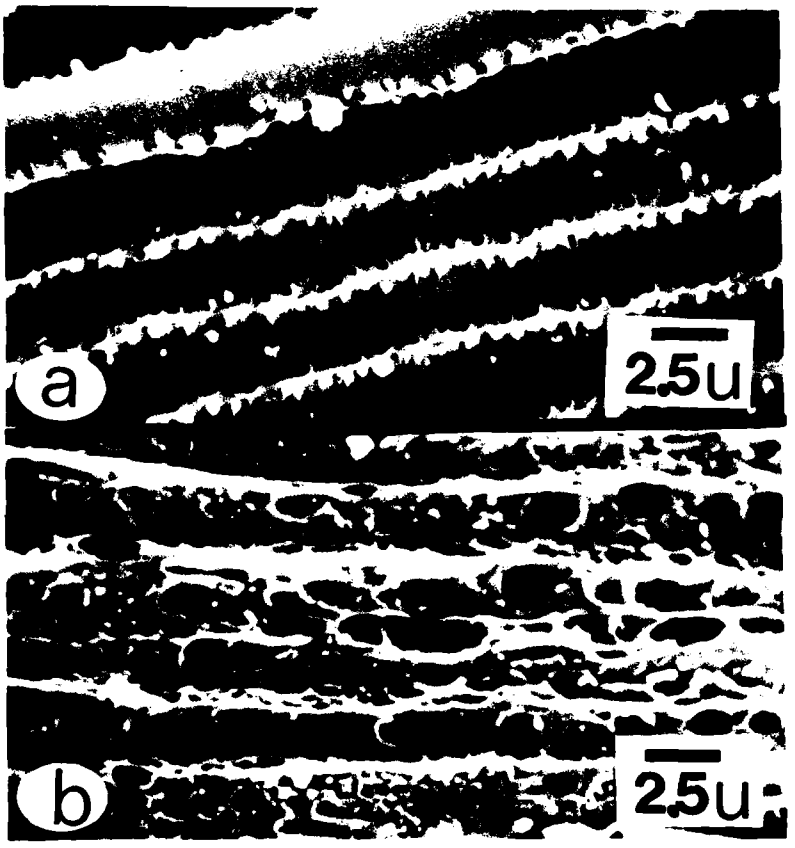
a
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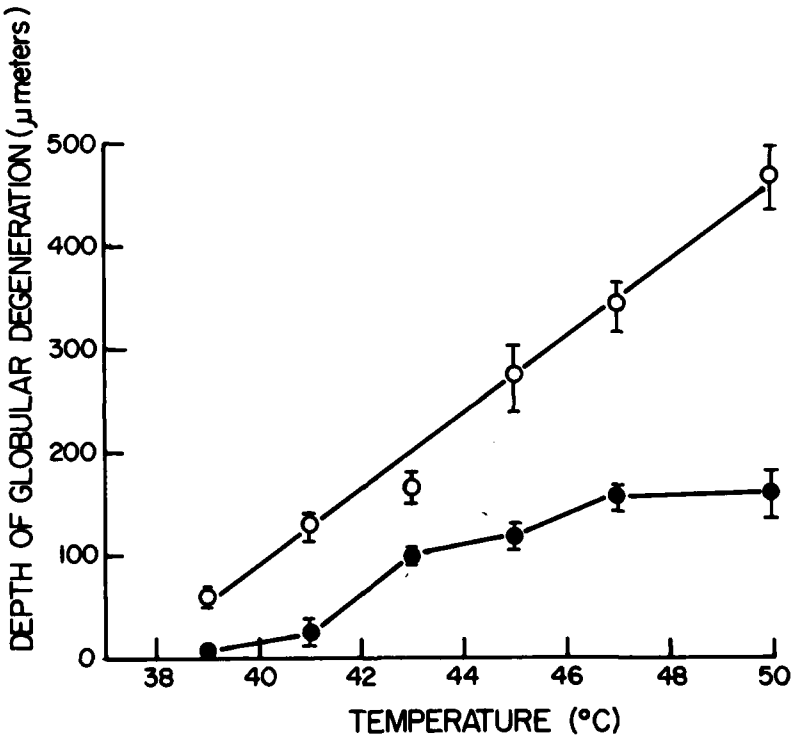
Fig. 5. Degree of globular degeneration as a function of incubation temperature (1 hr): ● maximum depth of degeneration at equator; O minimum depth of degeneration at anterior (or posterior) subcapsular region of lens. Bars represent standard error of the mean. Post-incubation period at 35.5°C was 47 hr.


DEPTH OF GLOBULAR DEGENERATION (μmeters)
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